

# Advertisement Call Variability in the Black-spined Toad *Bufo melanostictus* (Anura: Bufonidae) during the Breeding Season in Lishui, Zhejiang, China

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**Abstract** Advertisement calls play an important role in influencing female mate choice and are regarded as one of the key characters responsible for reproductive isolation and speciation events in frogs and toads. The black-spined toad, *Bufo melanostictus*, is one of the most common amphibians in Southeast Asia and is frequently observed in human habitations throughout its distributional range. The advertisement calls of the toad have not been well described. Here, we report the advertisement calls of this species from Lishui, Zhejiang, China during its breeding season. The advertisement calls of *B. melanostictus* are composed of single notes with a single harmonic frequency emitted sporadically. The call parameters recorded in this study were highly variable across recording times and with different body sizes. Dominant frequency was negatively correlated with body size. Comparing the calls of the *B. melanostictus* population from Lishui with the populations from five other localities (Bangkok, Thailand; Coorg, India; Burma, Myanmar; Bali, Indonesia; Halimun-Salak National Park, Indonesia), we found that call structure was similar among five different populations with no harmonics, while the Halimun-Salak National Park population contained two clear harmonics. Dominant frequency was similar between Bali (1.56 kHz) and Coorg (1.6 kHz) populations, while it was highly variable among other populations. Besides call duration, call interval and pulse/call rate may also be significantly different among the comparing populations. Pooling the available data, we observed some similarities and differences in call parameters among the six populations.

**Keywords** Anura, vocalization, *Bufo melanostictus*, acoustic parameters, advertisement call, artificial pond

## 1. Introduction

Acoustic signals are one of the most important forms of communication in anuran amphibians. On the basis of their function and environmental contexts, four types of calls were used to categorize: reciprocation calls, release calls, distress calls and advertisement calls (containing courtship calls, territorial calls, and encounter calls) (Duellman and Trueb, 1986). Of these, advertisement calls have been studied most extensively due to their unique species-specific characteristics (Gerhardt, 1994).

Advertisement calls play a very important role in female mate choice in frogs and toads, and are regarded as one of the key characters responsible for reproductive isolation and speciation events in this animal group (Wells, 1977; Cocroft and Ryan, 1995; Pough *et al.*, 2004; Hernández *et al.*, 2010; Bilate and Lack, 2011). Male anurans use vocalization to advertise their species identity, sex and location to females for the purpose of breeding (Márquez and Eekhout, 2006; Robillard *et al.*, 2006; Gerhardt and Brooks, 2009; Hernández *et al.*, 2010). Vocal behaviors also function in male–male competition, and are therefore subject to intra-sexual selection (Tobias *et al.*, 2004; Dubois *et al.*, 2009; Tobias *et al.*, 2010; Tobias *et al.*, 2011). Vocal signals are subject to natural selection when habitat affects signal transmission or leads to heightened risk of predation (Ryan *et al.*, 1982; Tobias *et al.*, 2011).

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The acoustic properties of different anuran calls are important in understanding the behavior and evolution of communications, and to provide the most comprehensive information on how to separate different call types between species (Krishna and Bosch, 2007).

So far, advertisement calls have been reported for approximately more than 20 species in China, including hylid, ranid, rhacophorid, microhylid, discoglossid, megophryid and pelobatid frogs (Jiang *et al.*, 1995; Jiang *et al.*, 2002; Xu *et al.*, 2005; Shen *et al.*, 2008; Yu and Zheng, 2009; Wei *et al.*, 2011; Chen *et al.*, 2011; Cui *et al.*, 2011; Cui *et al.*, 2012). From these studies, we know that there are two types of calls: audible calls (most of the studied species) and ultrasonic sounds (*Odorrana tormota*; Shen *et al.*, 2008). Comparing all the findings, we can conclude that the differences in songs across species should be correlated with some factors, such as physical structure (Whether a vocal sac is present or not), geographic habitat (such as elevation) and environment conditions (such as air temperature). The call traits were species-specific, and as such, the mating call is important for the female to distinguish conspecific from allospecific vocalizations (Jiang *et al.*, 2002). However, little is known about the call traits of the family Bufonidae. *Bufo melanostictus* (Anura: Bufonidae) is a bufonid with an extremely wide distribution range that covers most of Southeast Asia (Iskandar, 1998). In China, this species is mainly distributed in Sichuan, Yunnan, Guizhou, Zhejiang, Jiangxi, Hunan, Fujian, Taiwan, Guangdong, Guangxi and Hainan (Fei *et al.*, 2009). *B. melanostictus* is frequently found in human habitations throughout its distribution range. The ecology and natural history of *B. melanostictus* are poorly known, and its advertisement calls have not been well described in China. In this paper, we examined the advertisement calls of *B. melanostictus* from Lishui, Zhejiang, China during its breeding season. Because call properties of anurans are easily influenced proximately by various factors including air temperature, body temperature, body size and weight, and interactions among individuals (Giacoma *et al.*, 1997; Howard and Young, 1998; Navas and Bevier, 2001; Pröhl, 2003; Yu and Zheng, 2009), we tested the two hypotheses: 1) Call dominant frequency was negatively correlated with body size; and 2) some other call parameters such as call duration, call interval, call intensity, call duty cycle and call effort were highly variable across different recording time and with different body sizes. Finally, we compared the results obtained from the calls of this species with those described from other countries.

## 2. Materials and Methods

Because it is difficult to record individual toads from the field, we captured males ( $n = 66$ ) from one fish pond on the campus of Lishui University and took them to our laboratory also at Lishui University in early April 2011. According to their body sizes, at first we divided these toads into three groups: large-sized group (ranged from 60.91 mm to 70.97 mm,  $n = 22$ ), medium-sized group (from 53.92 mm to 59.96 mm,  $n = 24$ ) and small-sized group (from 40.77 mm to 55.93 mm,  $n = 20$ ), and then we released the toads of each group into an artificial pond (3 m  $\times$  2 m  $\times$  1 m) respectively, where some stones were placed. The three ponds were separated from each others by a distance of 30 m to mitigate sound interferences among the ponds. The water depth was approximate 20 cm. Following the method of Wei *et al.* (2011), at midnight (00:00 h) and twilight (18:00 h), we recorded advertisement calls emitted by each group using a Sony IC recorder (ICD-SX950) with an external directional microphone held at an approximate distance of 0.8 m from the water surface, with a sampling frequency resolution of 22050 Hz and 16 bit resolution. Concurrently, we also measured the air temperature from approximately 5 cm above the water surface of the ponds using a thermometer (HOBO U12-008, USA). The air temperature was approximately 22.0°C (21.4–22.2°C) at the two recording time points. After audio recording, toads were individually measured for body size (snout-vent length, SVL) to the nearest 0.1 mm with a dial caliper (Shanghai Medical Laser Company), weighed to the nearest 0.1 g using an electric scale (Jinnuo Balance Instrument Co., Ltd., Jinhua, China), and then released into the site where they were captured. Both SVL and body mass were significantly different among the three groups (Table 1).

Recording files were transferred with Cool Edit PRO (Syntrillium Software Corporation, USA) and saved as wav format. Calls were then analyzed with BatSound Pro (Pettersson Elektronik AB) for windows. Audiospectrograms were produced with the following parameters: FFT (Faster fourier transformation) = 512, sampling frequency resolution of 22050 Hz, Hanning window, time expansion  $\times 1$ . The sonogram, oscillogram and power spectrum were also performed in BatSound Pro for windows. Twenty calls in each call sequence within a sound file were analyzed, and terminology of calls followed Wei *et al.* (2011) and Leary *et al.* (2008): call duration (ms), call interval (ms), call intensity (dB), duty cycle (calculated by call duration/call duration plus

**Table 1** Characteristics of advertisement calls of *Bufo melanostictus*.

Parameters	Large-sized group		Medium-sized group		Small-sized group	
	00:00 h (No. = 400)	18:00 h (No. = 400)	00:00 h (No. = 400)	18:00 h (No. = 400)	00:00 h (No. = 280)	18:00 h (No. = 320)
Call duration (ms)	115.32 ± 0.30	100.19 ± 0.22	105.22 ± 0.29	92.87 ± 0.23	100.05 ± 0.25	90.38 ± 0.31
Call interval (ms)	192.95 ± 1.00	222.21 ± 1.25	171.06 ± 0.80	179.35 ± 0.96	230.03 ± 1.06	254.47 ± 1.46
Call intensity (dB)	19.82 ± 0.14	18.72 ± 0.14	20.14 ± 0.14	19.91 ± 0.13	21.00 ± 0.13	19.54 ± 0.13
Dominant frequency (kHz)	1.35 ± 0.11	1.35 ± 0.14	1.40 ± 0.13	1.46 ± 0.15	1.51 ± 0.14	1.48 ± 0.13
Duty cycle (%)	43.50 ± 35.73	39.87 ± 36.72	43.00 ± 33.36	39.73 ± 32.83	36.52 ± 36.11	34.84 ± 39.04
Call rate (calls/ms)	3.05 ± 0.28	2.79 ± 0.24	3.74 ± 0.13	3.41 ± 0.24	2.35 ± 0.28	2.62 ± 0.22
Call effort (calls/ms)	1.35 ± 0.32	1.12 ± 0.32	1.62 ± 0.24	1.37 ± 0.30	0.87 ± 0.34	0.92 ± 0.31
Snout-vent length (mm)	66.07 <sup>a</sup> ± 0.04 (n = 22)		57.27 <sup>b</sup> ± 0.04 (n = 24)		50.86 <sup>c</sup> ± 0.07 (n = 20)	
Body mass (g)	21.68 <sup>a</sup> ± 0.19 (n = 22)		14.46 <sup>b</sup> ± 0.10 (n = 24)		10.18 <sup>c</sup> ± 0.20 (n = 20)	

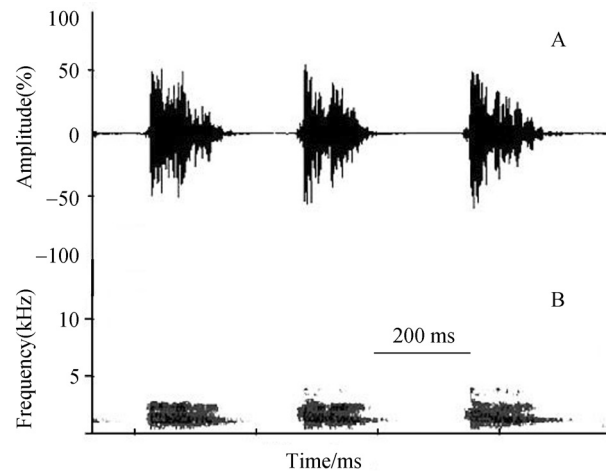
Types with different superscripts differ significantly (Tukey's test,  $\alpha = 0.05$ ,  $a > b > c$ ). No.: Call number; n: Sample size.

call interval; %), dominant frequency (DF, Hz), call rate (calls/ms) and call effort (calls/ms, duty cycle  $\times$  call rate). As amplitude was lower at the beginning than the remaining of the call sequence, the first 10 calls of every call sequence were removed from the analysis following a previous study (Pröhl, 2003). Comparative data from other geographic locations (countries) were obtained from the literature: Bangkok, Thailand (Heyer, 1971); Coorg, India (Hampson and Bennet, 2002); Burma, Myanmar (Wogan *et al.*, 2003); Bali, Indonesia (Márquez and Eekhout, 2006); Halimun-Salak National Park, Indonesia (Kurniati *et al.*, 2010).

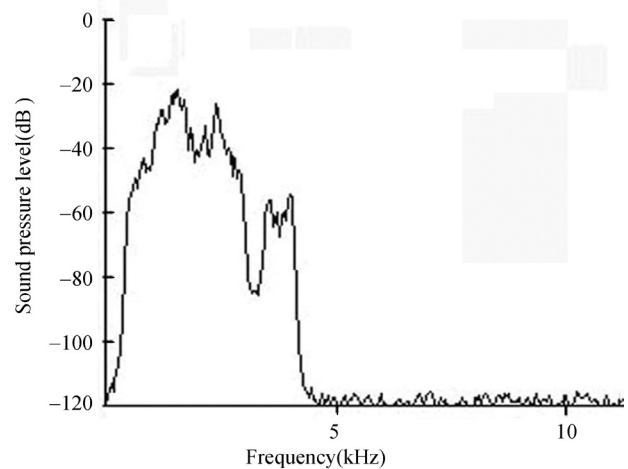
Statistical analyses were performed with the Statistica software package (version 5.0 for PC). All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variances. Data were analyzed with one-way and two-way ANOVA. All values were presented as mean  $\pm$  standard error (SE), and the significance level ( $\alpha$ ) was set at 0.05.

### 3. Results

According to our observations in the field for *B. melanostictus*, adult calls often lasted for an extended period of time (midnight and twilight) mutually separated by an interval distance of approximately 0.5–1.0 m. In this study, 66 adult males were captured from their chorus arena and divided into three groups, and we found SVL and body mass were both significantly different among them (One-way ANOVA,  $P < 0.05$ ; Table 1). Oscillograms, spectrograms and power spectrums of advertisement calls are shown in Figure 1 and Figure 2. The advertisement calls of this species are composed of single notes with a single harmonic frequency (Figure 1). The descriptions of each call parameter recorded from different recording time points and body sizes are shown



**Figure 1** Advertisement calls of *Bufo melanostictus*. A: Oscillograms; and B: Spectrograms from three calls.



**Figure 2** Power spectrum from the three calls described in Figure 1.

in Table 1.

To examine the effects of recording time and body size on the advertisement calls, we conducted a two-way ANOVA for call parameters for each group. For recording time within each group, call duration, call intensity,

duty cycle and call effort produced at midnight (I) were always larger than those produced in twilight (II) (two-way ANOVA,  $P < 0.05$ ), except for call interval (two-way ANOVA,  $P < 0.05$ ; Table 2). Dominant frequencies in each body size group were not significantly different between the two recording times (two-way ANOVA,  $P = 0.14$ ; Table 1 and Table 2). For body size, call durations were significantly different among the three groups (two-way ANOVA,  $P < 0.0001$ ; Table 2). Call duration in the large-sized group was greater than that in the other two groups (two-way ANOVA,  $P < 0.0001$ ; Table 2). Dominant frequency was also significantly different among the three groups (two-way ANOVA,  $P < 0.0001$ ; Table 2). Moreover, we found that dominant frequency was negatively correlated with body size, namely, larger individuals showed a lower dominant frequency (two-way ANOVA,  $P < 0.0001$ ; Table 1 and Table 2). Call interval was smaller in medium-sized group and larger in small group (two-way ANOVA,  $P < 0.0001$ ; Table 1 and Table 2). Call rate and call effort were significantly different among the three groups: small group was the lowest, large group was the second, and medium group was the highest (two-way ANOVA,  $P < 0.0001$ ; Table 2). However, we found no significant difference in the interaction of time  $\times$  size (two-way ANOVA, all  $P > 0.05$ ; Table 2). Combined to data mentioned as above, we found that the calls recorded during this study were highly variable across recording time and with different body sizes.

#### 4. Discussion

Based on our observations in the field, many males of *B. melanostictus* were found in wet habitats after the first heavy rainfall in early April of the study year, and most were calling with a long duration time. In Lishui, this species may display opportunistic behavior, with breeding mainly occurring during days with rainfall, as described for *Proceratophrys melanopogon* (Feio *et al.*, 2008).

Based on population data from six geographic locations (countries; including the present work), the

advertisement calls of *B. melanostictus* were highly variable across different locations. In the description of the Bangkok population (Heyer, 1971), the author recorded only eight calls from three individuals. These toads produced long trill calls with a duration ranging from 4 to 30 seconds and a pulse rate of 13.2 pulses per second at 25.0°C (air temperature). The dominant frequency of advertisement calls ranged from 1.0 kHz to 1.7 kHz ( $n = 3$ ,  $n_{\text{calls}} = 8$ ), with no harmonics. No more call parameters were provided in the work. The advertisement calls of *B. melanostictus* from the Coorg population were previously described by Hampson and Bennet (2002). They reported that the average duration of the calls was 40 seconds, and the average dominant frequency was 1.6 kHz. The average pulse rate and other parameters were not given for the Coorg population. Advertisement calls of *B. melanostictus* from the Burma population (Wogan *et al.*, 2003) had larger variability compared with the two former populations. They reported that the calls of *B. melanostictus* contained a series of notes made up of a variable number of pulses. The call dominant frequency recorded at 22.0°C (air temperature) was over 3.0 kHz, possessing an average SVL of 77.9 mm. The advertisement calls of *B. melanostictus* from the Bali population were described by Márquez and Eekhout (2006), with the average SVL, call duration, call pulse rate and dominant frequency of males being 64.2 (56–72) mm ( $n = 5$ ), 771.6 (678.9–933.0) ms ( $n_{\text{calls}} = 57$ ), 42.1 (40.6–51.4) pulses ( $n_{\text{calls}} = 57$ ) and 1555.2 (1433.5–1684.5) Hz ( $n_{\text{calls}} = 57$ ), respectively. Air temperature was 28°C–29°C. The call dominant frequency was similar (1.56 kHz vs 1.6 kHz) to that reported for the Coorg population (Hampson and Bennet, 2002). The sound of *B. melanostictus* from the Halimun-Salak National Park population consisted of two clear harmonics: the first one at 1450 Hz and the second at 2900 Hz, both having very small bandwidth (Kurniati *et al.*, 2010). However, no more data was given from their work.

In this work, we found that call frequency was negatively correlated with body size. Larger individuals

**Table 2** Statistical results of call parameters for factorial design recording time, body size and interaction of recording time  $\times$  body size using ANOVA procedure.

Parameters	Time	Size	Time $\times$ size
Call duration (ms)	$F_{1, 2194} = 110.83$ ; $P < 0.0001$ , I > II	$F_{2, 2194} = 39.85$ ; $P < 0.0001$ , L > M > S	$F_{2, 2194} = 1.72$ ; $P = 0.180$
Call interval (ms)	$F_{1, 2194} = 4.02$ ; $P < 0.046$ , I < II	$F_{2, 2194} = 13.48$ ; $P < 0.0001$ , M < L < S	$F_{2, 2194} = 0.41$ ; $P = 0.661$
Call intensity (dB)	$F_{1, 2194} = 65.98$ ; $P < 0.0001$ I > II	$F_{2, 2194} = 28.05$ ; $P < 0.0001$ , L < M = S	$F_{2, 2194} = 10.16$ ; $P < 0.0001$
Dominant frequency (kHz)	$F_{1, 2194} = 2.22$ ; $P = 0.14$	$F_{2, 2194} = 100.76$ ; $P < 0.0001$ , L < M < S	$F_{2, 2194} = 10.01$ ; $P < 0.0001$
Duty cycle (%)	$F_{1, 2194} = 21.84$ ; $P < 0.0001$ , I > II	$F_{2, 2194} = 37.37$ ; $P < 0.0001$ , L = M > S	$F_{2, 2194} = 0.84$ ; $P = 0.433$
Call rate (calls/ms)	$F_{1, 103} = 0.64$ ; $P = 0.426$	$F_{2, 103} = 100.76$ ; $P < 0.0001$ , S < L < M	$F_{2, 103} = 10.01$ ; $P = 0.167$
Call effort (calls/ms)	$F_{1, 103} = 3.94$ ; $P < 0.049$ , I > II	$F_{2, 103} = 21.61$ ; $P < 0.0001$ , S < L < M	$F_{2, 103} = 1.68$ ; $P = 0.192$

I: 00:00 h; II: 18:00 h; L: Large-sized group; M: Medium-sized group; S: Small-sized group. Significant level,  $\alpha = 0.05$ .



generally showed a lower dominant frequency. In contrast, we found call duration was positively correlated with body size. Both call duration and dominant frequency were usually correlated with mating success (Welch *et al.*, 1998; Pröhl, 2003). Comparing among the six populations, *B. melanostictus* produced two types of call structures. The call structures were similar among five populations (Bangkok, Coorg, Burma, Bali and Lishui) with no harmonics, while the Halimun-Salak National Park population contained two clear harmonics. Dominant frequencies were also highly variable among the populations (1.0–1.7 kHz in Bangkok; 3.0 kHz in Burma; 1.35 kHz, 1.40–1.46 kHz and 1.48–1.51 kHz in larger-sized, medium-sized and small-sized groups in Lishui, respectively), except the comparison of Bali (1.56 kHz) and Coorg (1.6 kHz) (Márquez and Eekhout, 2006). Likewise, call durations were significantly different among the populations, with the longest duration (40 seconds) found in the Bangkok population and the shortest duration (90 ms) found in the small-sized group in this study. Other call indexes, such as call pulse, were also different among the different populations. Pooling the available data, we can conclude that call traits (call structure, dominant frequency and call duration) of *B. melanostictus* recorded from different geographic locations were highly variable. This may be caused by some proximate factors such as different body sizes and air temperatures. However, SVL data from each population was not collected and it was thus unable to examine the relationship between call traits (e. g., call duration, call frequency, call interval, pulse rate) and SVL. From the available data, we found that SVL was largest in the Bali population and smallest in the small-sized group in Lishui. Moreover, as we did not collect body mass data from each population, we were unable to examine the relationship between call traits and body mass for the six populations. On the other hand, all the call data was recorded at different air temperatures, which could also conspicuously influence the call traits, especially the dominant frequency (Pröhl, 2003; Yu and Zheng, 2009). Aside from these factors, equipment, methods and sound-analysis software used also differed in each study. In any work, the comparison of call traits suggests both similarities (call structure) and differences (dominant frequency, call duration, call interval pulse/call rate) between the populations recorded. However, population traits (including call traits) can be affected by many factors, such as climate, microhabitat, gene flow and population history. Therefore, call traits may be affected by one or a combination of these factors.

Comparing all call traits for the six populations, we find that some significant differences did occur among these populations (e. g., call structure, dominant frequency, call duration, pulse/call rate). Why do such large differences exist? How were these differences formed? Further work (for example, molecular survey) should help us better understand why the variations occurred in the populations with large distribution areas.

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